510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

k123253

B. Purpose for Submission:

New device

C. Measurand:

1,25-Dihydroxy Vitamin D

D. Type of Test:

Quantitative, competitive chemiluminescent immunoassay

E. Applicant:

Immunodiagnostic Systems Ltd.

F. Proprietary and Established Names:

IDS iSYS 1,25 Dihydroxy Vitamin D
IDS iSYS 1,25 Dihydroxy Vitamin D Control Set
IDS iSYS 1,25 Dihydroxy Vitamin D Calibration Verifier

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 Vitamin D Test System	Clinical Chemistry (75)
JJX	Class I, reserved	21 CFR 862.1660 Quality Control Material	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   The IDS-iSYS 1,25 Dihydroxy Vitamin D assay is intended for the quantitative determination of 1,25 dihydroxyvitamin D levels in serum and plasma on the IDS-iSYS Multi-Discipline Automated System. Results of the 1,25 Dihydroxy Vitamin D are used in the assessment of vitamin D sufficiency.

   The IDS-iSYS 1,25 Dihydroxy Vitamin D Control Set is used for quality control of the IDS-iSYS 1,25 Dihydroxy Vitamin D assay on the IDS-iSYS Multi-Discipline Automated System

   The IDS-iSYS 1,25 Dihydroxy Vitamin D Calibration Verifier is a device intended for medical purposes for use in the quantitative verification of calibration of the IDS-iSYS 1,25 Dihydroxy Vitamin D assay when performed on the IDS-iSYS Multi-Discipline Automated Analyzer.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use with the IDS-iSYS Multi-Discipline Automated System (IS-310400)

I. Device Description:

The IDS-iSYS 1,25-Dihydroxy Vitamin D kit is an assay system intended for the purification of 1,25 dihydroxy vitamin D in human serum or plasma by offline immunopurification followed by quantitative determination on the IDS-iSYS Multi-Discipline Automated System. The components of the assay kit are described below.

IDS-iSYS 1,25-Dihydroxy Vitamin D kit

IDS-iSYS 1,25-Dihydroxy Vitamin D kit contains the immunoextraction kit, reagent cartridge, two (2) calibrators (A&B) and a mini CD.

The Immunoextraction kit contains the following reagents: 100 immunocapsules containing mouse monoclonal antibody to 1,25D linked to solid phase particles in suspension with vitamin D binding protein inhibitor (SORB), a delipidation reagent (REAG1), a solution of dextran sulphate and magnesium chloride, an elution reagent (REAG2), ethanol, an assay buffer (BUF) with MOPS buffer containing bovine serum

albumin with 0.01% sodium azide.

The reagent cartridge contains magnetic particles (MP), coated with streptavidin in a phosphate buffer containing bovine serum albumin and <0.1% sodium azide as preservative , a conjugate 1,25D labeled with an acridinium ester derivative in a phosphate buffer containing bovine serum albumin with <0.1% sodium azide as preservative (CONJ), ; Anti-1,25D polyclonal antibody labeled with biotin (Ab-BIOT), in a phosphate buffer containing sheep proteins with 0.01% sodium azide as preservative, and a wash buffer (BUFD).

Two calibrators are provided for use with the kit. IDS-iSYS 1,25 Dihydroxy Vitamin D Calibrator A (19.0 – 29.0 pg/mL) and Calibrator B (100.0 – 130.0 pg/mL) are provided lyophilized in MOPS buffer containing bovine serum albumin, 1,25Dihydroxy Vitamin D and <1.0% sodium azide as preservative .

IDS-iSYS 1,25-Dihydroxy Vitamin D kit Control kit

The IDS-iSYS 1,25-Dihydroxy Vitamin D Control set contains control sets (CTL1 & CTL2), extraction control sets (CTL3 & CTL4) and a mini CD. The controls (CTL1 & CTL2) are lyophilized 1,25D in buffered matrix with >0.1% (w/w) sodium azide as a preservative. The extraction controls are used to verify the validity of the sample results and must be used in parallel to the patient sample in the pretreatment procedure (extraction). Extraction controls (CTL3 & CTL4) are lyophilized 1,25D in human serum buffered with >0.1% (w/w) sodium azide as a preservative.

The IDS-iSYS 1,25 Dihydroxy Vitamin D Calibration Verifier

The IDS-iSYS 1,25 Dihydroxy Vitamin D Calibration Verifier set contains four levels of different concentrations of 1,25 D. Calibration verifiers are lyophilized MOPS buffer containing bovine serum albumin, 1,25D and sodium azide as preservative (<1.0%). 2 vials each of Cal. Ver. 0 – 3 (1.2mL per vial) are provided with the kit.

Human material used in the preparation of the product above has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Immunodiagnostic Systems Gamma-B 1, 25-Dihydroxy Vitamin D and Control Set

   Immunodiagnostic Systems Gamma-B 1,25-Dihydroxy Vitamin D Calibration Verifiers

2. Predicate 510(k) number(s):

k042519

k122141

3. Comparison with predicate:

Parameter	**Predicate Device (k042519)**	**Candidate device**
Intended Use	For the quantitative determination of 1,25-dihydroxyvitamin D (1,25D) in human serum or plasma.	Same
Measurand	1,25-dihydroxyvitamin D	Same
Sample matrix	Serum or plasma	Same
Antibodies	Monoclonal mouse anti-1,25D antibody for the purification and polyclonal sheep anti-25D antibody for the assay	Same
Sample volume	500 µL	500 µL
Method of detection	Radioactivity using Anti-sheep IgG coupled to cellulose solid phase and ^{125}I label	Chemilumninescence using magnetic-particle solid phase and acridinium label
Calibration	Full standard curve to be run with each assay run.	User –initiated 2 point calibration to adjust the batch related master curve. Performed every 14 days. The system stores the calibration for the interval specified in the kit IFU.
Quality Control	Requires two serum based extraction quality control samples. The QC samples are supplied with the Kits.	Requires two buffer based assay controls to validate the calibration and two serum based extraction quality control samples to verify the validity of the sample results. The IDS-iSYS 1,25 Dihydroxy Vitamin D Control Set supplied
Measuring Range	6 – 208 pg/mL	7.5 – 210.0 pg/mL
Reference range	20.2 – 63.0 pg/mL	26.1 – 95.0 pg/mL
Assay Duration	>24 hrs	~ 5.5 hrs

Controls		
Parameter	**Predicate Device (k042519)**	**Candidate device**
Intended Use	Used for the quality control of the 1,25-Dihydroxy Vitamin D assay	Same
Format	Lyophilized	Same
Stability	Store at 2-8ºC until expiration date	Same
Analyte	1,25-Dihydroxy Vitamin D	Same
Matrix	Serum based material	Assay controls are buffered matrix containing 1,25D. Extraction controls are buffered human serum matrix containing 1,25D

Calibration Verifiers		
Performance	**Predicate Device(k122141)**	**New device**
Intended Use	Used for the quantitative verification of calibration of the IDS-iSYS IGF-I assay.	Used for the quantitative verification of calibration of the IDS-iSYS 1,25-Dihydroxy Vitamin D assay.
Format	Lyophilized	Same
Stability	Store at 2-8ºC until expiration date	Same
Analyte	IGF-I	1,25-Dihydroxy Vitamin D
Matrix	Buffered protein	Bovine serum with sodium azide preservative

K. Standard/Guidance Document Referenced (if applicable):

CLSI C28-A2 How to Define and Determine Reference Intervals in the Clinical Laboratory
CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
CLSI EP7-A2 Inference Testing in Clinical Chemistry
CLSI EP9-A Method Comparison and Bias Estimation Using Patient Samples
CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantification
CEN 13640 Stability Testing of In Vitro Diagnostic Reagents

L. Test Principle:

The IDS-iSYS 1,25-Dihydroxy Vitamin D kit is a complete assay system for the purification of 1,25D in patient samples by immunopurification followed by quantitation by one-site chemiluminescent immunoassay on the automated IDS-iSYS instrument.

Patient samples are to be delipidated and 1,25D extracted from potential cross-reactants by incubation for 3 hours with a highly specific solid phase monoclonal anti-1,25D. 500 µL of the patient serum or plasma and the extraction controls (included in the The IDS-iSYS 1,25-Dihydroxy Vitamin D Control kit) are individually mixed with delipidation reagent and vortex and centrifuged. The calibrators and controls (CTL1, CTL2) do not undergo this step. Next the delipidated sample (150µL) undergoes immunoextraction. 150µL of delipidated sample or extraction controls are individually added to an immunocapsule which contains a gel containing a monoclonal anti-1,25D antibody. Rotation of the immunocapsule allows the binding of 1,25-dihydroxyvitamin D (1,25D) to the monoclonal antibody. The gel is washed to remove potential cross-reactants and the 1,25D eluted with ethanol. Eluates are reconstituted. Immunopurified samples are placed into the IDS-iSYS sample rack; the sample rack is then loaded on the IDS-iSYS system. 120 µL of the reconstituted immunopurified samples are incubated with the biotinylated sheep anti-1,25D antibody. The 1,25D-acridinium conjugate is then added which competes for antibody binding sites. Streptavidin coated magnetic particles are then added and following a further incubation step, the particles are washed to remove unbound materials. Following the addition of trigger reagents, a flash chemiluminescent reaction is initiated. The light signal is measured by the photomultiplier as Relative Light Units (RLU) and is inversely proportional to the amount of 1,25D present in the samples.

M. Performance Characteristics (if/when applicable):

All performance evaluations were conducted with the IDS-iSYS Multi-Discipline Automated System

1. Analytical performance:

   a. Precision/Reproducibility:

   The sponsor evaluated the precision of the IDS iSYS 1,25 Dihydroxy Vitamin D assay according to the CLSI EP5-A guideline. To assess reproducibility samples were assayed in duplicate, twice a day, for 20 days for an n=80. 4 controls (assay controls and extraction controls) and 4 patient samples were used in the precision study. All serum based samples underwent the imunoextraction process. Three IDS-iSYS Multi-Discipline Automated Systems were used (one per each reagent batch) and operated by a total of four personnel during the study. Precision results using one representative lot are summarized in the table below:

ID	Mean (pg/mL)	n	Within-run		Total	
			SD	CV%	SD	CV%
Sample 1	17.0	80	2.1	12.2%	3.1	18.0%
Sample 2	60.9	80	4.2	6.9%	6.6	10.8%
Sample 3	127.8	80	6.5	5.1%	13.6	10.6%
Sample 4	147.9	80	6.2	4.2%	16.3	11.0%
CTL1	37.2	80	2.4	6.3%	3.3	8.8%
CTL2	98.6	80	3.9	3.9%	7.1	7.2%
Extraction CTL3	44.9	80	3.3	7.4%	5.3	11.9%
Extraction CTL4	106.4	76	5.9	5.5%	10.9	10.2%

b. Linearity/assay reportable range:

The sponsor evaluated linearity according to the CLSI EP6-A guideline. Spiked serum sample with high 1,25 Dihydroxy vitamin D was used in the study. The high sample was diluted with low charcoal stripped, lipid stripped serum to achieve nine dilutions for the linearity study. All samples (11 totals) were tested in duplicate, covering the entire claimed measuring range (4.5 pg/mL to 210 mg/dL) of the 1,25 D assay.
The linear regression generated was: 1.01x+ 1.67 R^2=1.00.

The results of the study support the sponsor's claim that the assay is linear across the measuring range of 7.5 to 210 pg/mL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability

The ISD-iSYS 1,25D assay has been standardized against in-house reference standards. The calibrators and controls are manufactured using an internal in-house procedure and an internal reference standard.. The concentration of a 1st primary internal reference calibrator set was calculated by UV quantification using molar extinction coefficient 19000 at an absorbance of 264 nm.

Value assignments

The calibrators are value assigned by running duplicates in multiple assays on one instrument and must meet the internal specification. The assay controls are value assigned with the IDS-iSYS 1,25-Dihydroxy Vitamin D reagent with an on-board 2 point calibration. The assay controls are run in triplicate in multiple assays on at least three analyzers. The extraction controls are run in triplicate in multiple assays on at

least three analyzers. The extraction controls undergo the immuno-extraction process in each run. The control ranges are then calculated as the mean ± 3 standard deviations with an internal specification for acceptance.

The value assignment of the calibration verifiers is performed the same way as the kit assay controls.

The 1,25D calibrators have the following target ranges:

Calibrator A = 19.0 – 29.0 pg/mL
Calibrator B = 100.0 – 130.0 pg/mL

The 1, 25D Calibration verifiers have the following target ranges:

Calibration Verifiers 0 = below measuring range
Calibration Verifiers 1 = 20.0 – 30.0 pg/mL
Calibration Verifiers 2 = 85.0 – 115.0 pg/mL
Calibration Verifiers 3 = 195.0 – 240.0 pg/mL

The 1,25 D controls have the following target ranges:

CTL1= 29.0 – 41.0 pg/mL
CTL2= 77.0 – 104.0 pg/mL
CTL3= 29.0 – 41.0 pg/mL
CTL4= 77.0 – 104.0 pg/mL

Stability

Stability of IDS-iSYS 1,25-Dihydroxy Vitamin D assay cartridges, IDS iSYS 1,25 Dihydroxy Vitamin D calibrators, IDS iSYS 1,25 Dihydroxy Vitamin D controls, and calibration verifiers were assessed using real-time and accelerated stability studies. Protocols and acceptance criteria were reviewed and found to be acceptable to support the cartridge open-vial stability claim of 28 days when stored at the recommended storage temperatures of 2 - 8 °C. For shelf-life stability, the sponsor claimed that the controls and calibration verifiers are stable for 28 days at - 20 °C with a maximum of 2 freeze/thaw and the calibrators are stable for 28 days when stored at - 20 °C. Once reconstituted, the on-board stability of the calibration verifiers are 3 hours at 2 - 8 °C and extraction controls are 90 minutes. The extraction controls may also be stored at either 2 - 8 °C or -20 °C for 2 days after reconstitution.

c. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined based on the CLSI EP17-A.

LoB: For the determination of LoB, 10 replicates of calibrator A were run together with 3 replicates of calibrator B-G. The assay measurements were performed over multiple assay runs with one run per day using 3 lots of reagents to collect a minimum number of 60 measurements as required by the guideline. Two lots of calibrator sets and 2 analyzers were used over the LoB study. One analyzer was used for one reagent batch. Sponsor determined that LoB = mean (blank) + [1.645 x SD (blank)] supporting the sponsor's claim of LoB = 2.8 pg/mL.

LoD: The LoD was tested using 6, low charcoal lipid stripped serum and diluted serum samples in 6.0 – 12.7 pg/mL concentration range with each reagent batch. A total of 3 reagent lots were used for the measurement. Each sample was run in duplicate over 6 assay runs. Each sample underwent the entire immunoextraction process prior to measuring on the iSYS Multi-Discipline automated system analyzer. Each sample was analyzed in replicates and the LoD was calculated as:

LoD = LoB + 1.645 x pooled SD. Sponsor determined that the LoD = 5.5 pg/mL

LoQ was calculated when the % CVof the measured concentration on the iSYS was plotted against the measured concentration. The LOQ is the concentration which yields an inter-assay precision of ≤20 %. Sponsor determined that the LoQ = 7.5 pg/mL

The measuring range of the 1,25 D assay is 7.5 to 210 pg/mL.

e. Analytical specificity:

Interference study:

To determine potential interference in the specific detection of 1,25-Dihydroxy Vitamin D, two base serum samples at two different clinically relevant concentrations of 1,25-Dihydroxy Vitamin D (32 pg/mL and 165 pg/mL) were spiked with the potential interference substances. The interference materials were each spiked into both samples. One reagent batch was used for the interference testing. The differences observed between the mean spiked and control sample values were examined and assessed according to acceptance criteria. The sponsor defined significant interference as <10% difference between the spiked and the control samples.

Interference was calculated using:

$$\% \text{ Interference} = \frac{(\text{mean spiked value} - \text{mean control value})}{\text{mean control value}} \times 100$$

The following are the highest concentration tested without significant interference:

Triglycerides	1000 mg/dL
Hemoglobin	200 mg/dL
Bilirubin	20 mg/dL
Albumin	9.1 g/dL
Red Blood Cells	0.4%
Cholesterol	300 mg/dL
Biotin	300 nM
HAMA	1000 ng/mL
Rheumatoid Factor	2079 IU/mL

Since hemolyzed samples will affect the results, the sponsor has the following limitations in their labeling:

"Hemolyzed samples should not be used with this assay."

Cross reactivity study:

To assess cross-reactivity, nine potential cross reactants were spiked into analyte free serum and tested on the candidate device. A reference preparation was performed side by side with the cross-reactant using the 1,25(OH)2D3 antigen. The %cross-reactivity was calculated from the ED50 results of the displacement curves of the serial dilutions using the formula below and the % cross reactivity results are summarized in the table below.

$(ED50_{1,25(OH)2D3}/ED50_{cross\text{-}reactant}) \times 100$

Potential Cross-reactant	Cross-reactivity
1,25(OH)2D3	97.6%
1,25(OH)2D2	75%
1,24,25,(OH)3D3	92%
25(OH)D3	0.0015%
25(OH)D2	0.0009%
24,25(OH)2D3	0.006%
24,25(OH)2D2	0.005%
Epi-25(OH)D3	< 0.0015%
Alfacalcidol	0.04%

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor performed a method comparison study according to the CLSI EP9-A2 guideline. Results from the candidate device were compared to those obtained using the predicate device. A total of 121 serum samples with concentrations across the reportable range of the assay were used for the method comparison study. Six normal samples were diluted to achieve more samples close to the bottom of the assay range. All samples were serum and the sample range tested was 10.7 to 197.5 pg/mL. The following are the results from the Passing Bablok regression analysis: $y = 1.00x – 2.8$; $r = 0.95$.

Slope = 1.00 (95% CI = 0.93 to 1.07)
Intercept = -2.8 pg/mL (95% CI = -6.3 pg/mL to 0.4 pg/mL).
Calculation of the correlation coefficient using linear regression was r = 0.95.

b. Matrix comparison:

To assess the effect of anticoagulants on the performance of the IDS-iSYS 1,25-Dihydroxy Vitamin D assay the results obtained from plasma sample tube were compared to results obtained from samples drawn into the following tubes: serum (without additives), serum separator tubes (SST), potassium EDTA plasma, lithium heparin plasma and sodium heparin plasma. A total of 19 paired tubes were tested using 3 lots of reagents against the plain serum tube. Sample range tested between 12.6 to 210 pg/mL. The results from Passing-Bablok analysis are presented below:

Serum separator tubes: $y = 1.00 \times \text{serum} + 1.26$ pg/mL $R^2 = 0.99$
K2 EDTA plasma tubes: $y = 1.01 \times \text{serum} + 0.57$ pg/mL $R^2 = 1.00$
Li Heparin plasma tubes: $y = 1.02 \times \text{serum} + 0.23$ pg/mL $R^2 = 0.99$,
Na Heparin plasma tubes: $y = 1.01 \times \text{serum} – 1.30$ pg/mL $R^2 = 0.99$,

The sponsor concluded that the assay could be performed using serum (standard serum tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples. Samples should be separated as soon as possible after collection.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

The reference interval was determined by using 119 serum samples from apparently healthy male and female (62 male, 59 female) subjects, aged 18-60, collected in standard serum sample tubes. Ethnicity was as follows: Caucasian 66 (54.5%) and Hispanic 55 (45.5%). The 95% reference interval for 1,25-Dihydroxy Vitamin D was calculated by a non-parametric method following the CLSI C28-A2 guideline.

Normal Adults: 26.1 – 95.0 pg/mL.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.